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<b>(21) International Application Number:</b> PCT/US94/11440 <b>(22) International Filing Date:</b> 7 October 1994 (07.10.94)  <b>(30) Priority Data:</b> 08/134,078                      8 October 1993 (08.10.93)                      US  <b>(71) Applicant (for all designated States except US):</b> THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; Suite 2-100, 2024 E. Monument Street, Baltimore, MD 21205 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LEE, Se-Jin [US/US]; 6711 Chokeberry Road, Baltimore, MD 21209 (US). CUNNINGHAM, Noreen [US/US]; 1400 Mimosa Lane, Silver Spring, MD 20904 (US).  <b>(74) Agents:</b> WETHERELL, John, R., Jr. et al.; Spensley Horn Jubas & Lubitz, 5th floor, 1880 Century Park East, Los Angeles, CA 90067 (US).		<b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>  <div style="text-align: center; font-size: 2em; font-weight: bold; margin-top: 100px;">BEST AVAILABLE COPY</div>
<b>(54) Title:</b> GROWTH DIFFERENTIATION FACTOR-10  <b>(57) Abstract</b>  Growth differentiation factor-10 (GDF-10) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-10 polypeptide and polynucleotide sequences.		

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## GROWTH DIFFERENTIATION FACTOR-10

### BACKGROUND OF THE INVENTION

#### 1. *Field of the Invention*

5 The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF- $\beta$ ) superfamily, which is denoted, growth differentiation factor-10 (GDF-10).

#### 2. *Description of Related Art*

10 The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the  
15 imaginal disks (Padgett, *et al.*, *Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al.*, *Cell*, 51:861-867, 1987), the activins (Mason, *et al.*, *Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos  
20 (Thomsen, *et al.*, *Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al.*, *J. Biol. Chem.*, 265:13198, 1990). The TGF- $\beta$ s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis,

and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

5 The proteins of the TGF- $\beta$  family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 10 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. For most of the family members that have been studied, the homodimeric species has been found to be biologically 15 active, but for other family members, like the inhibins (Ling, *et al.*, *Nature*, 321:779, 1986) and the TGF- $\beta$ s (Cheifetz, *et al.*, *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

20 Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function and allow development of effective diagnostic and therapeutic regimens.

## SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-10, a polynucleotide sequence which encodes the factor, and antibodies which are immunoreactive with the factor. This factor  
5 appears to relate to various cell proliferative disorders, especially those involving those involving uterine, nerve, bone, and adipose tissue.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of uterine, nerve, or fat origin and which is associated with GDF-10. In another embodiment, the invention provides  
10 a method for treating a cell proliferative disorder by suppressing or enhancing GDF-10 activity.

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## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows expression of GDF-10 mRNA in adult tissues.

FIGURE 2 shows nucleotide and predicted amino acid sequence murine GDF-10. Consensus N-glycosylation signals are denoted by plain boxes.

FIGURE 3 shows the alignment of the C-terminal sequences of GDF-10 with other members of the TGF- $\beta$  superfamily. The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize alignment.

FIGURE 4 shows amino acid homologies with different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus.

FIGURE 5 shows an alignment of the C-terminal sequences of human (top lines) and murine (bottom lines) GDF-10.

FIGURE 6 shows an autoradiogram of labeled secreted proteins synthesized by 293 cells transfected with a pcDNA1 vector into which the GDF-10 cDNA was inserted in either the antisense (lanes 1 and 2) or sense (lanes 3 and 4) orientation.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-10 and a polynucleotide sequence encoding GDF-10. GDF-10 is expressed at highest levels in uterus and fat and at lower levels in other tissues, such as brain. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of uterine, nerve, or fat origin which is associated with GDF-10 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder by using an agent which suppresses or enhances GDF-10 activity.

The TGF- $\beta$  superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-10 protein of this invention and the members of the TGF- $\beta$  family, indicates that GDF-10 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-10 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

The expression of GDF-10 in uterine and fat tissue suggests a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to contraception, fertility, pregnancy, and cell proliferative diseases. Abnormally low levels of the factor may be indicative of impaired function in the uterus while abnormally high levels may be indicative of hypertrophy, hyperplasia, or the presence of ectopic tissue. Hence, GDF-10 may be useful in detecting not only



primary and metastatic neoplasms of uterine origin but in detecting diseases such as endometriosis as well. In addition, GDF-10 may also be useful as an indicator of developmental anomalies in prenatal screening procedures.

5 Several members of the TGF- $\beta$  superfamily possess activities suggesting possible applications for the treatment of cell proliferative disorders, such as cancer. In particular, TGF- $\beta$  has been shown to be potent growth inhibitor for a variety of cell types (Massague, *Cell* 49:437, 1987). MIS has been shown to inhibit the growth of human  
10 endometrial carcinoma tumors in nude mice (Donahoe, *et al.*, *Ann. Surg.* 194:472, 1981), and inhibin  $\alpha$  has been shown to suppress the development of tumors both in the ovary and in the testis (Matzuk, *et al.*, *Nature*, 360:313, 1992). GDF-10 may have similar activity and may therefore be useful as an anti-proliferative agent, such as for the  
15 treatment of endometrial cancer or endometriosis.

Many of the members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been shown to have marked effects on the formation of collagen and causes of striking angiogenic response in the newborn mouse (Roberts, *et al.*, *Proc. Nat'l Acad. Sci., USA* 83:4167,  
20 1986). The BMP's can induce new bone growth and are effective for the treatment of fractures and other skeletal defects (Glowacki, *et al.*, *Lancet*, 1:959, 1981; Ferguson, *et al.*, *Clin. Orthoped. Relat. Res.*, 227:265, 1988; Johnson, *et al.*, *Clin Orthoped Relat. Res.*, 230:257, 1988). Based on the high degree of homology between GDF-10 and  
25 BMP-3, GDF-10 may have similar activities and may be useful in repair of tissue injury caused by trauma or burns for example.



GDF-10 may play a role in regulation of the menstrual cycle or regulation of uterine function during pregnancy, and therefore, GDF-10, anti-GDF-10 antibodies, or antisense polynucleotides may be useful either in contraceptive regimens, in enhancing the success of *in vitro* fertilization procedures, or in preventing premature labor.

Certain members of this superfamily have expression patterns or possess activities that relate to the function of the nervous system. For example, one family member, namely GDNF, has been shown to be a potent neurotrophic factor that can promote the survival of dopaminergic neurons (Lin, *et al.*, *Science*, 260:1130). Another family member, namely dorsalin, is capable of promoting the differentiation of neural crest cells (Baster, *et al.*, *Cell*, 73:687). The inhibins and activins have been shown to be expressed in the brain (Meunier, *et al.*, *Proc. Nat'l Acad. Sci.*, USA, 85:247, 1988; Sawchenko, *et al.*, *Nature*, 334:615, 1988), and activin has been shown to be capable of functioning as a nerve cell survival molecule (Schubert, *et al.*, *Nature*, 344:868, 1990). Another family member, namely GDF-1, is nervous system-specific in its expression pattern (Lee, *Proc. Nat'l Acad. Sci.*, USA, 88:4250, 1991), and certain other family members, such as Vgr-1 (Lyons, *et al.*, *Proc. Nat'l Acad. Sci.*, USA, 86:4554, 1989; Jones *et al.*, *Development*, 111:581, 1991), OP-1 (Ozkaynak, *et al.*, *J. Biol. Chem.*, 267:25220, 1992), and BMP-4 (Jones, *et al.*, *Development*, 111:531, 1991), are also known to be expressed in the nervous system. By analogy GDF-10 may have applications in the treatment of neurodegenerative diseases or in maintaining cells or tissues in culture prior to transplantation.

The expression of GDF-10 in adipose tissue also raises the possibility of applications for GDF-10 in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. In this regard, TGF- $\beta$

has been shown to be a potent inhibitor of adipocyte differentiation *in vitro* (Ignotz and Massague, *Proc. Natl. Acad. Sci., USA* 82:8530, 1985).

5 The term "substantially pure" as used herein refers to GDF-10 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-10 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-10 polypeptide can  
10 also be determined by amino-terminal amino acid sequence analysis. GDF-10 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-10 remains. Smaller peptides containing the biological activity of GDF-10 are included in the invention.

The invention provides polynucleotides encoding the GDF-10 protein.  
15 These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-10. It is understood that all polynucleotides encoding all or a portion of GDF-10 are also included herein, as long as they encode a polypeptide with GDF-10 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated  
20 polynucleotides. For example, GDF-10 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-10 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are  
25 specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-10 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a cDNA sequence for GDF-10 which is 2322 base pairs in length and contains an open reading frame beginning with a methionine codon at nucleotide 126. The encoded polypeptide is 476 amino acids in length with a molecular weight of about 52.5 kD, as determined by nucleotide sequence analysis. The GDF-10 sequence contains a core of hydrophobic amino acids near the N-terminus, suggestive of a signal sequence for secretion. GDF-10 contains four potential N-glycosylation sites at asparagine residues 114, 152, 277, and 467. GDF-10 contains several potential proteolytic processing sites. Cleavage most likely occurs following arginine 365, which would generate a mature fragment of GDF-10 predicted to be 111 amino acids in length and have an unglycosylated molecular weight of about 12.6kD, as determined by nucleotide sequence analysis. One skilled in the art can modify, or partially or completely remove, the glycosyl groups from the GDF-10 protein using standard techniques. Therefore the functional protein or fragments thereof of the invention includes glycosylated, partially glycosylated and unglycosylated species of GDF-10.

The C-terminal region of GDF-10 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily. The GDF-10 sequence contains most of the residues that are highly conserved in other family members. Among the known family mammalian TGF- $\beta$  family members, GDF-10 is most homologous to BMP-3 (83% sequence identity beginning with the first conserved cysteine residue). GDF-10 also shows significant homology to BMP-3 (approximately 30% sequence identity) in the pro-region of the molecule. Based on these sequence comparisons, GDF-10 and BMP-3 appear to define a new subfamily within the larger superfamily.

Minor modifications of the recombinant GDF-10 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-10 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or  
5 may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-10 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the  
10 development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-10 biological activity.

The nucleotide sequence encoding the GDF-10 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the  
15 replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for  
20 another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with  
25 the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to:

1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-10 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).



The development of specific DNA sequences encoding GDF-10 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in

DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

5 A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-10 peptides having at least one epitope, using antibodies specific for GDF-10. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-10 cDNA.

10 DNA sequences encoding GDF-10 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the  
15 term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

~~In the present invention, the GDF-10 polynucleotide sequences may be~~  
inserted into a recombinant expression vector. The term "recombinant  
expression vector" refers to a plasmid, virus or other vehicle known in  
20 the art that has been manipulated by insertion or incorporation of the  
GDF-10 genetic sequences. Such expression vectors contain a  
promoter sequence which facilitates the efficient transcription of the  
inserted genetic sequence of the host. The expression vector typically  
contains an origin of replication, a promoter, as well as specific genes  
25 which allow phenotypic selection of the transformed cells. Vectors  
suitable for use in the present invention include, but are not limited to  
the T7-based expression vector for expression in bacteria (Rosenberg,



et al., *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably  
5 linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-10 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences  
10 having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal region of GDF-10 is expressed from  
15 a cDNA clone containing the entire coding sequence of GDF-10. Alternatively, the C-terminal portion of GDF-10 can be expressed as a fusion protein with the pro- region of another member of the TGF- $\beta$  family or co-expressed with another pro- region (see for example, Hammonds, et al., *Molec. Endocrin.* 5:149, 1991; Gray, A., and Mason,  
20 A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after  
25 exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also  
5 be cotransformed with DNA sequences encoding the GDF-10 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform  
10 eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by  
conventional means including preparative chromatography and  
15 immunological separations involving monoclonal or polyclonal antibodies.

The invention includes antibodies immunoreactive with GDF-10  
~~polypeptide or functional fragments thereof.~~ Antibody which consists  
essentially of pooled monoclonal antibodies with different epitopic  
20 specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 1975). The term antibody as used  
in this invention is meant to include intact molecules as well as  
25 fragments thereof, such as Fab and F(ab')<sub>2</sub>, which are capable of binding an epitopic determinant on GDF-10.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. The term "cell-proliferative disorder" also includes situations in which a normally occurring process could be enhanced or suppressed for clinical benefit; an example of such a process would be fracture healing. Malignant cells (i.e. cancer) develop as a result of a multistep process. The GDF-10 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, cells in uterine or adipose tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-10 could be considered susceptible to treatment with a GDF-10 suppressing reagent. One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of uterine or adipose tissue which comprises contacting an anti-GDF-10 antibody with a cell suspected of having a GDF-10 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-10 is labeled with a compound which allows detection of binding to GDF-10. For purposes of the invention, an antibody specific for GDF-10 polypeptide may be used to detect the level of GDF-10 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is uterine or fat tissue. The level of GDF-10 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-10-associated cell proliferative disorder. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or

immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples  
5 of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be  
10 done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

15 The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, ~~amylases~~, ~~natural and modified celluloses~~, polyacrylamides, agaroses  
20 and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

25 There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those

of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

5 Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

10 In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having  
15 the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background.  
20 Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and  
25 extent of disease of the individual. Such dosages may vary, for



example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are  $^{111}\text{In}$ ,  $^{97}\text{Ru}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{89}\text{Zr}$ , and  $^{201}\text{Tl}$ .

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements

-20-

which are particularly useful in such techniques include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Cr}$ , and  $^{56}\text{Fe}$ .

5 The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-10-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the  
10 GDF-10-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-10-associated disease in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal  
15 cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-10, nucleic acid sequences that interfere with GDF-10 expression at the translational level can be used. This approach utilizes, for example,  
20 antisense nucleic acid and ribozymes to block translation of a specific GDF-10 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule  
25 (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with



the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target  
5 GDF-10-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA  
10 restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-  
15 specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. ~~*Tetrahymena*-type-ribozymes~~ recognize sequences which are four bases  
in length, while "hammerhead"-type ribozymes recognize base  
20 sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to  
25 shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by GDF-10 protein. Such

therapy would achieve its therapeutic effect by introduction of the GDF-10 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-10 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-10 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the GDF-10 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to  $\Psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-10 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu$ m can encapsulate a substantial percentage of an aqueous

buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al.*, *Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-10 primarily in uterine and adipose tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these and other tissues. Such applications include treatment of cell proliferative disorders involving these and other tissues, including bone. In addition, GDF-10 may be useful in various gene therapy procedures.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.





CA). DNA was prepared from bacterial colonies carrying individual subclones and sequenced. Of 11 clones that were sequenced, 9 corresponded to BMP-3, and two represented a novel sequence, which was designated GDF-10.

5

## EXAMPLE 2

### EXPRESSION PATTERN AND SEQUENCE OF GDF-10

To determine the expression pattern of GDF-10, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. 2.5 micrograms of twice polyA-selected RNA prepared from each tissue were electrophoresed on formaldehyde gels, blotted and probed with GDF-10. As shown in Figure 1, the GDF-10 probe detected an mRNA expressed at highest levels in uterus, fat, and brain.

A murine uterus cDNA library consisting of  $3 \times 10^6$  recombinant phage was constructed in lambda ZAP II and screened with a probe derived from the GDF-10 PCR product. The entire nucleotide sequence of the longest of 7 hybridizing clones is shown in Figure 2. Consensus N-glycosylation signals are denoted by plain boxes. Numbers indicate nucleotide position relative to the 5' end. The 2322 bp sequence contains a long open reading frame beginning with a methionine codon at nucleotide 126 and potentially encoding a protein 476 amino acids in length with a molecular weight of 52.5 kD. The predicted GDF-10 amino acid sequence contains a hydrophobic N-terminal region, suggestive of a signal sequence for secretion, four potential N-linked glycosylation sites at asparagine residues 114, 152, 277, and 467 and a putative proteolytic processing site at amino acid 365. Cleavage of the GDF-10 precursor at this site would generate a mature GDF-10



protein 111 amino acids in length with a predicted unglycosylated molecular weight of 12.6 kD.

The C-terminal region of GDF-10 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily (Figure 3). Figure 3 shows the alignment of the C-terminal sequences of GDF-10 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 1991), murine GDF-3 and GDF-9 (McPherron and Lee, *J. Biol. Chem.* 268:3444, 1993), human BMP-2 and 4 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human Vgr-1 (Celeste, *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, *et al.*, *EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-2 (Ozkaynak, *et al.*, *J. Biol. Chem.*, 267:25220-25227, 1992), human BMP-3 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human MIS (Cate, *et al.*, *Cell*, 45:685-698, 1986), human inhibin alpha,  $\beta$ A, and  $\beta$ B (Mason, *et al.*, *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), murine nodal (Zhou, *et al.*, *Nature*, 361:543-547, 1993), human TGF- $\beta$ 1 (Derynck, *et al.*, *Nature*, 316:701-705, 1985), human TGF- $\beta$ 2 (deMartin, *et al.*, *EMBO J.*, 6:3673-3677, 1987), and human TGF- $\beta$ 3 (ten Dijke, *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

GDF-10 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing.

FIGURE 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities calculated from the first conserved cysteine to the C-terminus. In this region, GDF-10 is most homologous to BMP-3 (83% sequence identity).

### EXAMPLE 3

#### ISOLATION OF HUMAN GDF-10

To isolate human GDF-10, a human uterus cDNA library consisting of  $16.2 \times 10^6$  recombinant phage was constructed in lambda ZAP II and screened with a murine GDF-10 probe. From this library, 20 hybridizing clones were isolated. Partial nucleotide sequence analysis of the longest clone showed that human and murine GDF-10 are highly homologous; the predicted amino acid sequences are 97% identical beginning with the first conserved cysteine residue following the predicted cleavage site (Figure 5).

### EXAMPLE 4

#### SECRETION OF GDF-10 BY MAMMALIAN CELLS

To determine whether GDF-10 is secreted by mammalian cells, the GDF-10 cDNA was cloned into the pcDNA1 expression vector and transfected into 293 cells. Following DNA transfection, the cells were metabolically labeled with a mixture of [ $^{35}$ S]-cysteine and [ $^{35}$ S]-methionine, and labeled secreted proteins were analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Figure 6, additional bands were detected in cells transfected with a sense GDF-10 construct compared to an antisense control construct. The presence of multiple

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protein species most likely indicates that 293 cells are capable of proteolytically processing GDF-10. Hence, these data suggest that GDF-10 is secreted by these cells and that GDF-10 is cleaved, as predicted from the cDNA sequence.

- 5 Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

(ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-10

5 (iii) NUMBER OF SEQUENCES: 26

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: Los Angeles  
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(E) COUNTRY: USA  
(F) ZIP: 90067

(v) COMPUTER READABLE FORM:

15 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

20 (A) APPLICATION NUMBER: PCT  
(B) FILING DATE: 07-OCT-1994  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

25 (A) NAME: LISA A. HAILE, PH.D.  
(B) REGISTRATION NUMBER: P-38,347  
(C) REFERENCE/DOCKET NUMBER: FD-3054 PCT

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(A) TELEPHONE: (619) 455-5100  
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## 30 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: NSC1

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10 CCGGAATTCA ARGTINGAYTT YGCNGAYATH GGNTGG

36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: NSC2

20

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCTC CANGCRCARC TYTCNACNGT CAT

33

25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

-33-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- 5 (vii) IMMEDIATE SOURCE:  
(B) CLONE: NSC3

- (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1..33

- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGGAATTCTC CANGCRCANG AYTCNACNGT CAT

33

(2) INFORMATION FOR SEQ ID NO:4:

- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2322 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- 20 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Murine GDF-10

- (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 126..1553

-34-

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	TGGGGTCATC CGGGCTGTCC GAGTCCCACA GGGACAACTC CAGCCGCGGA CGAGGTGCAC	60
	AGCCAACACT GAGCCCTCCT TGTCTGTTCT CCTGGGCTCA GACCCTTCAC CACCGTTACT	120
5	CAGCC ATG GCT CCA GGT CCT GCT CGG ATC AGC TTG GGG TCC CAG CTG Met Ala Pro Gly Pro Ala Arg Ile Ser Leu Gly Ser Gln Leu 1 5 10	167
	CTG CCC ATG GTG CCG CTG CTC CTG CTG CTG CGG GGC GCA GGC TGC GGC Leu Pro Met Val Pro Leu Leu Leu Leu Leu Arg Gly Ala Gly Cys Gly 15 20 25 30	215
10	CAC AGG GGC CCC TCA TGG TCC TCA TTG CCC TCG GCA GCT GCC GGT CTG His Arg Gly Pro Ser Trp Ser Ser Leu Pro Ser Ala Ala Ala Gly Leu 35 40 45	263
15	CAG GGG GAC AGG GAC TCC CAG CAG TCA CCC GGG GAC GCA GCA GCC GCT Gln Gly Asp Arg Asp Ser Gln Gln Ser Pro Gly Asp Ala Ala Ala Ala 50 55 60	311
	CTG GGC CCA GGC GCC CAG GAC ATG GTC GCT ATC CAC ATG CTC AGG CTC Leu Gly Pro Gly Ala Gln Asp Met Val Ala Ile His Met Leu Arg Leu 65 70 75	359
20	TAT GAG AAG TAC AAC CGA AGA GGT GCT CCA CCG GGA GGA GGC AAC ACC Tyr Glu Lys Tyr Asn Arg Arg Gly Ala Pro Pro Gly Gly Gly Asn Thr 80 85 90	407
	GTC CGA AGC TTC CGT GCC CGG CTG GAA ATG ATC GAC CAA AAG CCT GTG Val Arg Ser Phe Arg Ala Arg Leu Glu Met Ile Asp Gln Lys Pro Val 95 100 105 110	455
25	TAT TTC TTC AAC TTG ACT TCC ATG CAA GAC TCA GAA ATG ATC CTC ACA Tyr Phe Phe Asn Leu Thr Ser Met Gln Asp Ser Glu Met Ile Leu Thr 115 120 125	503
30	GCC GCC TTC CAC TTC TAC TCA GAA CCT CCA CGG TGG CCC CGG GCT GGT Ala Ala Phe His Phe Tyr Ser Glu Pro Pro Arg Trp Pro Arg Ala Gly 130 135 140	551
	GAG GTA TTC TGC AAG CCC CGA GCT AAG AAC GCA TCC TGC CGC CTC CTG Glu Val Phe Cys Lys Pro Arg Ala Lys Asn Ala Ser Cys Arg Leu Leu 145 150 155	599



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	ACC CCA GGG CTG CCT GCA CGC TTG CAC CTA ATC TTC CGC AGT CTT TCC	647
	Thr Pro Gly Leu Pro Ala Arg Leu His Leu Ile Phe Arg Ser Leu Ser	
	160 165 170	
5	CAG AAC ACC GCC ACT CAG GGG CTG CTC CGC GGG GCC ATG GCC CTG ACG	695
	Gln Asn Thr Ala Thr Gln Gly Leu Leu Arg Gly Ala Met Ala Leu Thr	
	175 180 185 190	
	CCT CCA CCA CGT GGC CTG TGG CAG GCC AAG GAC ATC TCC TCA ATC ATC	743
	Pro Pro Pro Arg Gly Leu Trp Gln Ala Lys Asp Ile Ser Ser Ile Ile	
	195 200 205	
10	AAG GCT GCC CGA AGG GAT GGA GAG CTG CTT CTC TCT GCT CAG CTG GAT	791
	Lys Ala Ala Arg Arg Asp Gly Glu Leu Leu Leu Ser Ala Gln Leu Asp	
	210 215 220	
	ACT GGG GAG AAG GAC CCC GGA GTG CCA CGG CCC AGT TCC CAC ATG CCC	839
	Thr Gly Glu Lys Asp Pro Gly Val Pro Arg Pro Ser Ser His Met Pro	
15	225 230 235	
	TAT ATC CTT GTC TAC GCC AAT GAC CTG GCC ATC TCC GAA CCC AAC AGT	887
	Tyr Ile Leu Val Tyr Ala Asn Asp Leu Ala Ile Ser Glu Pro Asn Ser	
	240 245 250	
	GTA GCA GTG TCG CTA CAG AGA TAC GAC CCA TTT CCA GCT GGA GAC TTT	935
20	Val Ala Val Ser Leu Gln Arg Tyr Asp Pro Phe Pro Ala Gly Asp Phe	
	255 260 265 270	
	GAG CCT GGA GCA GCC CCC AAC AGC TCA GCT GAT CCC CGC GTG CGC AGG	983
	Glu Pro Gly Ala Ala Pro Asn Ser Ser Ala Asp Pro Arg Val Arg Arg	
	275 280 285	
25	GCG GCT CAG GTG TCA AAA CCC CTG CAA GAC AAT GAA CTG CCG GGG CTG	1031
	Ala Ala Gln Val Ser Lys Pro Leu Gln Asp Asn Glu Leu Pro Gly Leu	
	290 295 300	
	GAT GAA AGA CCA GCG CCT GCC CTG CAT GCC CAG AAT TTC CAC AAG CAC	1079
30	Asp Glu Arg Pro Ala Pro Ala Leu His Ala Gln Asn Phe His Lys His	
	305 310 315	
	GAG TTC TGG TCC AGT CCT TTC CGG GCA CTG AAA CCC CGC ACG GCG CGC	1127
	Glu Phe Trp Ser Ser Pro Phe Arg Ala Leu Lys Pro Arg Thr Ala Arg	
	320 325 330	

-36-

	AAA GAC CGC AAG AAG AAG GAC CAG GAC ACA TTC ACC GCC GCC TCC TCT	1175
	Lys Asp Arg Lys Lys Lys Asp Gln Asp Thr Phe Thr Ala Ala Ser Ser	
	335                      340                      345                      350	
5	CAG GTG CTG GAC TTT GAC GAG AAG ACG ATG CAG AAA GCC AGG AGG CGG	1223
	Gln Val Leu Asp Phe Asp Glu Lys Thr Met Gln Lys Ala Arg Arg Arg	
	355                      360                      365	
	CAG TGG GAT GAG CCC CGG GTC TGC TCC AGG AGG TAC CTG AAG GTG GAT	1271
	Gln Trp Asp Glu Pro Arg Val Cys Ser Arg Arg Tyr Leu Lys Val Asp	
	370                      375                      380	
10	TTT GCA GAC ATC GGG TGG AAT GAA TGG ATC ATC TCT CCC AAA TCC TTT	1319
	Phe Ala Asp Ile Gly Trp Asn Glu Trp Ile Ile Ser Pro Lys Ser Phe	
	385                      390                      395	
	GAC GCC TAC TAC TGT GCT GGG GCC TGC GAG TTC CCC ATG CCC AAG ATT	1367
15	Asp Ala Tyr Tyr Cys Ala Gly Ala Cys Glu Phe Pro Met Pro Lys Ile	
	400                      405                      410	
	GTC CGC CCA TCC AAC CAT GCC ACC ATC CAG AGC ATC GTC AGA GCT GTG	1415
	Val Arg Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val	
	415                      420                      425                      430	
20	GGC ATT GTC CCT GGC ATC CCA GAG CCA TGC TGT GTT CCA GAC AAG ATG	1463
	Gly Ile Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Asp Lys Met	
	435                      440                      445	
	AAC TCC CTT GGA GTC CTT TTC CTG GAT GAA AAT CGG AAT GCG GTT CTG	1511
	Asn Ser Leu Gly Val Leu Phe Leu Asp Glu Asn Arg Asn Ala Val Leu	
	450                      455                      460	
25	AAG GTG TAC CCC AAT ATG TCC GTA GAG ACC TGT GCC TGT CGG	1553
	Lys Val Tyr Pro Asn Met Ser Val Glu Thr Cys Ala Cys Arg	
	465                      470                      475	
	TAAGATGGCT TCAAGATAGA AGACAGACCT GCTTCATCCC TGCCCTGCAG AGTGGCAATC	1613
	TTGGAGCCAG GGACTTGACT CGGGGAGGTT CCAGGTGCTA GACAGAGCTT ACAGGCAGCC	1673
30	CTGCTGGGAC CAAGAAAGAT CTGCCACCA CATCGCAATT CTTCACTTCT TCCGTGCTGG	1733
	TGGTAGCTCT GTAAAGACGT GTTGAGTTCC TGGAAGAAAT CTGGAATTAA CTGTGGTCTG	1793
	CAATTTGCCC ATCATCCCTG CCCACACTTT TCAAGGCCTA GAAATAACGT GTGTCCTCAA	1853

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ATGTCAACTC CAGGCATTTG TCCTCTCAA ACCTAGAAAG ACTATGCAA TCTTGGGGTA 1913  
 CTCCCCCCCC CCATGGCAGT TTAAATGCTG TTTTAAAACC CTCAGGCTGC ATTCTAGAAA 1973  
 CAGGGCCTAA CCCATGGCAC GAGTGAGTAT TTTCTCTTAC GTTTCAC TAC ACGTGCTTTT 2033  
 ATACATGCAG TATGCACATG TAATCACGGT TGATTTCTTC TTTTAATATA TGTATTTCTA 2093  
 5 TTTCAAAGCA AAACGGAGAG AGTCGATCCC ATCCCCTGCA GAGGTAATAA TGCAAGTTAG 2153  
 GTGTGGGTTG TCTAAGCATG TGTATGGAAA TAATACATAC AGTAATATGC TGAATACTA 2213  
 AAAAAGTAAC CAAGATTTTA TATTTTGTGTA AATTATACTT TGTATACTGT AGATTGTGAG 2273  
 TGTTCCTGTGT TTTTATGGAA AGCTAATAAA ~~TTAAAGGTGC~~ GGAGGTATC 2322

(2) INFORMATION FOR SEQ ID NO:5:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 476 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Pro Gly Pro Ala Arg Ile Ser Leu Gly Ser Gln Leu Leu Pro  
 1 5 10 15

Met Val Pro Leu Leu Leu Leu Arg Gly Ala Gly Cys Gly His Arg  
 20 25 30

20

Gly Pro Ser Trp Ser Ser Leu Pro Ser Ala Ala Ala Gly Leu Gln Gly  
 35 40 45

Asp Arg Asp Ser Gln Gln Ser Pro Gly Asp Ala Ala Ala Ala Leu Gly  
 50 55 60

25

Pro Gly Ala Gln Asp Met Val Ala Ile His Met Leu Arg Leu Tyr Glu  
 65 70 75 80

Lys Tyr Asn Arg Arg Gly Ala Pro Pro Gly Gly Gly Asn Thr Val Arg  
 85 90 95

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	Ser	Phe	Arg	Ala	Arg	Leu	Glu	Met	Ile	Asp	Gln	Lys	Pro	Val	Tyr	Phe	
				100					105					110			
	Phe	Asn	Leu	Thr	Ser	Met	Gln	Asp	Ser	Glu	Met	Ile	Leu	Thr	Ala	Ala	
			115					120					125				
5	Phe	His	Phe	Tyr	Ser	Glu	Pro	Pro	Arg	Trp	Pro	Arg	Ala	Gly	Glu	Val	
			130				135					140					
	Phe	Cys	Lys	Pro	Arg	Ala	Lys	Asn	Ala	Ser	Cys	Arg	Leu	Leu	Thr	Pro	
	145					150					155					160	
10	Gly	Leu	Pro	Ala	Arg	Leu	His	Leu	Ile	Phe	Arg	Ser	Leu	Ser	Gln	Asn	
					165					170					175		
	Thr	Ala	Thr	Gln	Gly	Leu	Leu	Arg	Gly	Ala	Met	Ala	Leu	Thr	Pro	Pro	
				180					185					190			
	Pro	Arg	Gly	Leu	Trp	Gln	Ala	Lys	Asp	Ile	Ser	Ser	Ile	Ile	Lys	Ala	
			195					200					205				
15	Ala	Arg	Arg	Asp	Gly	Glu	Leu	Leu	Leu	Ser	Ala	Gln	Leu	Asp	Thr	Gly	
			210				215					220					
	Glu	Lys	Asp	Pro	Gly	Val	Pro	Arg	Pro	Ser	Ser	His	Met	Pro	Tyr	Ile	
	225					230					235					240	
20	Leu	Val	Tyr	Ala	Asn	Asp	Leu	Ala	Ile	Ser	Glu	Pro	Asn	Ser	Val	Ala	
				245						250					255		
	Val	Ser	Leu	Gln	Arg	Tyr	Asp	Pro	Phe	Pro	Ala	Gly	Asp	Phe	Glu	Pro	
				260					265					270			
	Gly	Ala	Ala	Pro	Asn	Ser	Ser	Ala	Asp	Pro	Arg	Val	Arg	Arg	Ala	Ala	
			275					280				285					
25	Gln	Val	Ser	Lys	Pro	Leu	Gln	Asp	Asn	Glu	Leu	Pro	Gly	Leu	Asp	Glu	
			290				295					300					
	Arg	Pro	Ala	Pro	Ala	Leu	His	Ala	Gln	Asn	Phe	His	Lys	His	Glu	Phe	
	305					310					315					320	
30	Trp	Ser	Ser	Pro	Phe	Arg	Ala	Leu	Lys	Pro	Arg	Thr	Ala	Arg	Lys	Asp	
				325						330					335		

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Arg Lys Lys Lys Asp Gln Asp Thr Phe Thr Ala Ala Ser Ser Gln Val  
 340 345 350  
 Leu Asp Phe Asp Glu Lys Thr Met Gln Lys Ala Arg Arg Arg Gln Trp  
 355 360 365  
 5 Asp Glu Pro Arg Val Cys Ser Arg Arg Tyr Leu Lys Val Asp Phe Ala  
 370 375 380  
 Asp Ile Gly Trp Asn Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala  
 385 390 395 400  
 10 Tyr Tyr Cys Ala Gly Ala Cys Glu Phe Pro Met Pro Lys Ile Val Arg  
 405 410 415  
 Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Ile  
 420 425 430  
 Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Asp Lys Met Asn Ser  
 435 440 445  
 15 Leu Gly Val Leu Phe Leu Asp Glu Asn Arg Asn Ala Val Leu Lys Val  
 450 455 460  
 Tyr Pro Asn Met Ser Val Glu Thr Cys Ala Cys Arg  
 465 470 475

(2) INFORMATION FOR SEQ ID NO:6:

20

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 120 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

25

- (ii) MOLECULE TYPE: protein

- (vii) IMMEDIATE SOURCE:
- (B) CLONE: GDF-10

- (ix) FEATURE:

30

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..120

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

	Glu	Lys	Ser	Met	Gln	Lys	Ala	Arg	Arg	Arg	Gln	Trp	Asp	Glu	Pro	Arg
	1				5					10					15	
5	Val	Cys	Ser	Arg	Arg	Tyr	Leu	Lys	Val	Asp	Phe	Ala	Asp	Ile	Gly	Trp
				20				25						30		
	Asn	Glu	Trp	Ile	Ile	Ser	Pro	Lys	Ser	Phe	Asp	Ala	Tyr	Tyr	Cys	Ala
			35					40					45			
	Gly	Ala	Cys	Glu	Phe	Pro	Met	Pro	Lys	Ile	Val	Arg	Pro	Ser	Asn	His
	50						55					60				
10	Ala	Thr	Ile	Gln	Ser	Ile	Val	Arg	Ala	Val	Gly	Ile	Val	Pro	Gly	Ile
	65					70					75					80
	Pro	Glu	Pro	Cys	Cys	Val	Pro	Asp	Lys	Met	Asn	Ser	Leu	Gly	Val	Leu
				85						90					95	
	Phe	Leu	Asp	Glu	Asn	Arg	Asn	Ala	Val	Leu	Lys	Val	Tyr	Pro	Asn	Met
15				100					105					110		
	Ser	Val	Glu	Thr	Cys	Ala	Cys	Arg								
			115					120								

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: GDF-1

(ix) FEATURE:

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..123



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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly  
 1 5 10 15  
 Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp  
 5 20 25 30  
 His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln  
 35 40 45  
 Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro  
 50 55 60  
 Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro  
 10 65 70 75 80  
 Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile  
 85 90 95  
 Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr  
 15 100 105 110  
 Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg  
 115 120

## (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 118 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: GDF-3

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..118

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

[illegible]

(2) INFORMATION FOR SEQ ID NO:9:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-9

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..119
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

[illegible]

(2) INFORMATION FOR SEQ ID NO:10:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 118 amino ac

- (A) LENGTH: 118 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-2

(ix) FEATURE:

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..118

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

[illegible]

(2) INFORMATION FOR SEQ ID NO:11:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-4

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..118
```

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys  
 1 5 10 15  
 Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp  
 5 20 25 30  
 Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His  
 35 40 45  
 Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His  
 50 55 60  
 Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys  
 10 65 70 75 80  
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu  
 85 90 95  
 Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val  
 15 100 105 110  
 Glu Gly Cys Gly Cys Arg  
 115

## (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 119 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: Vgr-1

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..119

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

[illegible]

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: OP-1

(ix) **FEATURE:**

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..119



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

[illegible]

(2) INFORMATION FOR SEQ ID NO:14:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-5

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..119
```

(2) INFORMATION FOR SEQ ID NO:15:

(ii) - MOLECULE TYPE: protein

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..119
```

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln  
 1 5 10 15  
 Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp  
 5 20 25 30  
 Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu  
 35 40 45  
 Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His  
 50 55 60  
 Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro  
 10 65 70 75 80  
 Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr  
 85 90 95  
 Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val  
 15 100 105 110  
 Val Lys Ala Cys Gly Cys His  
 115

## (2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 120 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: BMP-3

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..120

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

	Glu	Gln	Thr	Leu	Lys	Lys	Ala	Arg	Arg	Lys	Gln	Trp	Ile	Glu	Pro	Arg
	1				5					10					15	
5	Asn	Cys	Ala	Arg	Arg	Tyr	Leu	Lys	Val	Asp	Phe	Ala	Asp	Ile	Gly	Trp
				20					25					30		
	Ser	Glu	Trp	Ile	Ile	Ser	Pro	Lys	Ser	Phe	Asp	Ala	Tyr	Tyr	Cys	Ser
			35					40						45		
	Gly	Ala	Cys	Gln	Phe	Pro	Met	Pro	Lys	Ser	Leu	Lys	Pro	Ser	Asn	His
		50					55					60				
10	Ala	Thr	Ile	Gln	Ser	Ile	Val	Arg	Ala	Val	Gly	Val	Val	Pro	Gly	Ile
	65					70					75					80
	Pro	Glu	Pro	Cys	Cys	Val	Pro	Glu	Lys	Met	Ser	Ser	Leu	Ser	Ile	Leu
					85					90					95	
	Phe	Phe	Asp	Glu	Asn	Lys	Asn	Val	Val	Leu	Lys	Val	Tyr	Pro	Asn	Met
15				100					105					110		
	Thr	Val	Glu	Ser	Cys	Ala	Cys	Arg								
			115					120								

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:

- (B) CLONE: MIS

(ix) **FEATURE:**

- (A) NAME/KEY: Protein  
(B) LOCATION: 1..116

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

[illegible]

(2) INFORMATION FOR SEQ ID NO:18:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 122 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin-alpha

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..122
```

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Leu Arg Leu Leu Gln Arg Pro Pro Glu Glu Pro Ala Ala His Ala  
 1 5 10 15  
 Asn Cys His Arg Val Ala Leu Asn Ile Ser Phe Gln Glu Leu Gly Trp  
 5 20 25 30  
 Glu Arg Trp Ile Val Tyr Pro Pro Ser Phe Ile Phe His Tyr Cys His  
 35 40 45  
 Gly Gly Cys Gly Leu His Ile Pro Pro Asn Leu Ser Leu Pro Val Pro  
 50 55 60  
 Gly Ala Pro Pro Thr Pro Ala Gln Pro Tyr Ser Leu Leu Pro Gly Ala  
 10 65 70 75 80  
 Gln Pro Cys Cys Ala Ala Leu Pro Gly Thr Met Arg Pro Leu His Val  
 85 90 95  
 Arg Thr Thr Ser Asp Gly Gly Tyr Ser Phe Lys Tyr Glu Thr Val Pro  
 15 100 105 110  
 Asn Leu Leu Thr Gln His Cys Ala Cys Ile  
 115 120

## (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 121 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: Inhibin-beta-A

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..121



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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg Arg Arg Arg Arg Gly Leu Glu Cys Asp Gly Lys Val Asn Ile Cys  
 1 5 10 15  
 Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn Asp  
 5 20 25 30  
 Trp Ile Ile Ala Pro Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly Glu  
 35 40 45  
 Cys Pro Ser His Ile Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe His  
 50 55 60  
 Ser Thr Val Ile Asn His Tyr Arg Met Arg Gly His Ser Pro Phe Ala  
 10 65 70 75 80  
 Asn Leu Lys Ser Cys Cys Val Pro Thr Lys Leu Arg Pro Met Ser Met  
 85 90 95  
 Leu Tyr Tyr Asp Asp Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln Asn  
 15 100 105 110  
 Met Ile Val Glu Glu Cys Gly Cys Ser  
 115 120

## (2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 120 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: Inhibin-beta-B

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..120

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	Arg	Ile	Arg	Lys	Arg	Gly	Leu	Glu	Cys	Asp	Gly	Arg	Thr	Asn	Leu	Cys
	1				5					10					15	
5	Cys	Arg	Gln	Gln	Phe	Phe	Ile	Asp	Phe	Arg	Leu	Ile	Gly	Trp	Asn	Asp
				20					25					30		
	Trp	Ile	Ile	Ala	Pro	Thr	Gly	Tyr	Tyr	Gly	Asn	Tyr	Cys	Glu	Gly	Ser
			35					40					45			
	Cys	Pro	Ala	Tyr	Leu	Ala	Gly	Val	Pro	Gly	Ser	Ala	Ser	Ser	Phe	His
		50					55					60				
10	Thr	Ala	Val	Val	Asn	Gln	Tyr	Arg	Met	Arg	Gly	Leu	Asn	Pro	Gly	Thr
	65					70					75				80	
	Val	Asn	Ser	Cys	Cys	Ile	Pro	Thr	Lys	Leu	Ser	Thr	Met	Ser	Met	Leu
					85					90					95	
15	Tyr	Phe	Asp	Asp	Glu	Tyr	Asn	Ile	Val	Lys	Arg	Asp	Val	Pro	Asn	Met
				100						105					110	
	Ile	Val	Glu	Glu	Cys	Gly	Cys	Ala								
				115				120								

## (2) INFORMATION FOR SEQ ID NO:21:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: Nodal

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Trp Gly Arg Arg Gln Arg Arg His His Leu Pro Asp Arg Ser Gln  
 1 5 10 15  
 Leu Cys Arg Arg Val Lys Phe Gln Val Asp Phe Asn Leu Ile Gly Trp  
 5 20 25 30  
 Gly Ser Trp Ile Ile Tyr Pro Lys Gln Tyr Asn Ala Tyr Arg Cys Glu  
 35 40 45  
 Gly Glu Cys Pro Asn Pro Val Gly Glu Glu Phe His Pro Thr Asn His  
 50 55 60  
 Ala Tyr Ile Gln Ser Leu Leu Lys Arg Tyr Gln Pro His Arg Val Pro  
 10 65 70 75 80  
 Ser Thr Cys Cys Ala Pro Val Lys Thr Lys Pro Leu Ser Met Leu Tyr  
 85 90 95  
 Val Asp Asn Gly Arg Val Leu Leu Glu His His Lys Asp Met Ile Val  
 15 100 105 110  
 Glu Glu Cys Gly Cys Leu  
 115

## (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 114 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: TGF-beta-1

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..114

(2) INFORMATION FOR SEQ ID NO:23:

(ii) MOLECULE TYPE: protein

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..114
```

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

[illegible]

(2) INFORMATION FOR SEQ ID NO:24:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 114 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: TGF-beta-3

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..114
```

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

[illegible]

(2) INFORMATION FOR SEQ ID NO:25:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 115 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Human GDF-10

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1..115
```



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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Lys Ala Arg Arg Lys Gln Trp Asp Glu Pro Arg Val Cys Ser Arg Arg  
 1 5 10 15  
 Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Asn Glu Trp Ile Ile  
 5 20 25 30  
 Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ala Gly Ala Cys Glu Phe  
 35 40 45  
 Pro Met Pro Lys Ile Val Arg Pro Ser Asn His Ala Thr Ile Gln Ser  
 50 55 60  
 Ile Val Arg Ala Val Gly Ile Ile Pro Gly Ile Pro Glu Pro Cys Cys  
 10 65 70 75 80  
 Val Pro Asp Lys Met Asn Ser Leu Gly Val Leu Phe Leu Asp Glu Asn  
 85 90 95  
 Arg Asn Val Val Leu Lys Val Tyr Pro Asn Met Ser Val Asp Thr Cys  
 15 100 105 110  
 Ala Cys Arg  
 115

## (2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:  
 20 (A) LENGTH: 115 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
 (B) CLONE: Murine GDF-10

(ix) FEATURE:  
 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..115

[illegible]

**CLAIMS**

1. Substantially pure growth differentiation factor-10 (GDF-10) and functional fragments thereof.
2. An isolated polynucleotide sequence encoding the GDF-10 polypeptide of claim 1.
3. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.
4. The polynucleotide of claim 3, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.
5. An expression vector including the polynucleotide of claim 2.
6. The vector of claim 5, wherein the vector is a plasmid.
7. The vector of claim 5, wherein the vector is a virus.
8. A host cell stably transformed with the vector of claim 5.
9. The host cell of claim 8, wherein the cell is prokaryotic.
10. The host cell of claim 8, wherein the cell is eukaryotic.
11. Antibodies reactive with the polypeptide of claim 1 or fragments thereof.

12. The antibodies of claim 11, wherein the antibodies are polyclonal.
13. The antibodies of claim 11, wherein the antibodies are monoclonal.
14. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 11 with a specimen of a subject suspected of having a GDF-10 associated disorder and detecting binding of the antibody.
15. The method of claim 14, wherein the cell is a uterine cell.
16. The method of claim 14, wherein the cell is a fat cell.
17. The method of claim 14, wherein the detecting is *in vivo*.
18. The method of claim 17, wherein the antibody is detectably labeled.
19. The method of claim 18, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, and an enzyme.
20. The method of claim 14, wherein the detection is *in vitro*.
21. The method of claim 20, wherein the antibody is detectably labeled.

22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.
23. A method of treating a cell proliferative disorder associated with expression of GDF-10, comprising contacting the cells with a reagent which suppresses the GDF-10 activity.
24. The method of claim 23, wherein the reagent is an anti-GDF-10 antibody.
25. The method of claim 23, wherein the reagent is a GDF-10 antisense sequence.
26. The method of claim 23, wherein the cell is a uterine cell.
27. The method of claim 23, wherein the cell is a fat cell.
28. The method of claim 23, wherein the reagent which suppresses GDF-10 activity is introduced to a cell using a vector.
29. The method of claim 28, wherein the vector is a colloidal dispersion system.
30. The method of claim 29, wherein the colloidal dispersion system is a liposome.

31. The method of claim 30, wherein the liposome is essentially target specific.
32. The method of claim 31, wherein the liposome is anatomically targeted.
33. The method of claim 31, wherein the liposome is mechanistically targeted.
34. The method of claim 33, wherein the mechanistic targeting is passive.
35. The method of claim 33, wherein the mechanistic targeting is active.
36. The method of claim 35, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.
37. The method of claim 36, wherein the protein moiety is an antibody.
38. The method of claim 28, wherein the vector is a virus.
39. The method of claim 38, wherein the virus is an RNA virus.
40. The method of claim 39, wherein the RNA virus is a retrovirus.
41. The method of claim 40, wherein the retrovirus is essentially target specific.

42. The method of claim 41, wherein the moiety for target specificity is encoded by a polynucleotide inserted into the retroviral genome.
43. The method of claim 42, wherein the moiety for target specificity is selected from the group consisting of a sugar, a glycolipid, and a protein.
44. The method of claim 43, wherein the protein is an antibody.



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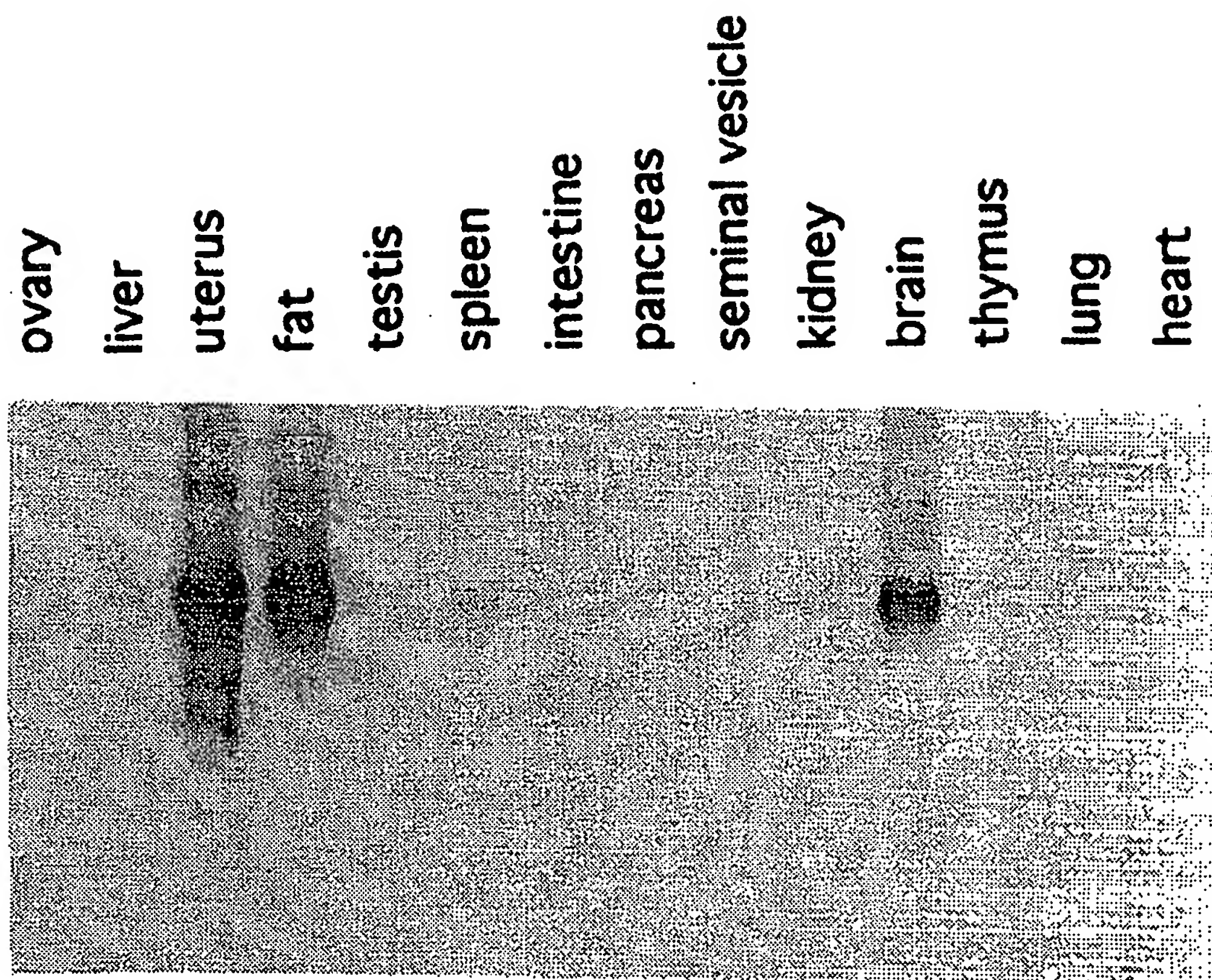


FIG. 1

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1 TGGGTATCCGGGCTGTCCGAGTCCACAGGGACAACCTCCAGCCGGACGAGGTGCAC 60  
 61 AGCCAAACACTGAGCCCTCTTGTCTGTCTCTCTGGGCTCAGACCCCTTACACCACCGTTACT 120  
 121 CAGCCATGGCTCCAGGTCTCTCGGATCAGCTTGGGGTCCAGCTGCTGCCCATGGTGC 180  
     M A P G P A R I S L G S Q L L P M V P  
 181 CGCTGCTCCTGCTGCGGGCGCAGGCTGGGCCACAGGGGCCCTCATGTCCTCAT 240  
     L L L L R G A G C G H R G P S W S L  
 241 TGCCCTCGGCAGCTGCCGGTCTGCAGGGGACAGGGACTCCAGCAGTCACCCGGGGACG 300  
     P S A A A G L Q G D R D S Q Q S P G D A  
 301 CAGCAGCCGCTTGCGCCAGGCGCCAGGACATGTCGCTATCCACATGCTCAGGCTCT 360  
     A A A L G P G A Q D M V A I H M L R L Y  
 361 ATGAGAAGTACAACCGAAGGTGCTCCACCGGAGGAGGCAACCCGTCCGAAGCTTCC 420  
     E K Y N R R G A P P G G G N T V R S F R  
 421 GTGCCCCGCTGGAATGATCGACCAAGCCCTGTGTATTCTTCAACTTGACTTCCATGC 480  
     A R L E M I D Q K P V Y F F N L T S M Q  
 481 AAGACTCAGAAATGATCCTCAGAGCGGCTTCCACTTCTACTCAGAACCTCCACGGTGC 540  
     D S E M I L T A A F H F Y S E P P R W P  
 541 CCCGGGCTGGTGAGGTATTCTGCAAGCCCCGAGCTAAGAACGCATCCTGCCGCCCTCCTGA 600  
     R A G E V F C K P R A K N A S C R L L T  
 601 CCCAGGGCTGCTGCAGGCTTGACCTAATCTCCGAGTCTTCCAGAACACCGCCA 660  
     P G L P A R L H L I F R S L S Q N T A T  
 661 CTCAGGGGCTGCTCCGGGGCCATGGCCCTGAGCCCTCCACCACGTGGCCTGTGGCAGG 720  
     Q G L L R G A M A L T P P P R G L W Q A  
 721 CCAAGGACATCTCCTCAATCATCAAGGCTGCCCGAAGGATGGAGAGTGCTTCTCTCTG 780  
     K D I S S I I K A A R R D G E L L S A  
 781 CTCAGCTGGATACTGGGAGAAGGACCCCGAGTGCCACGGCCAGTCCACATGCCCT 840  
     Q L D T G E K D P G V P R P S S H M P Y  
 841 ATATCCTTGTACGCCAATGACCTGGCCATCTCCGAACCCAAACAGTGTAGCAGTGTGCG 900  
     I L V Y A N D L A I S E P N S V A V S L  
 901 TACAGAGATACGCCCATTTCCAGCTGGAGACTTTGAGCCTGGAGCAGCCCCCAACAGCT 960  
     Q R Y D P F P A G D F E P G A A P N S S  
 961 CAGCTGATCCCGGCTGCGCAGGGGCTCAGGTGTCAAAACCCCTGCAAGACAATGAAC 1020  
     A D P R R R A A Q V S K P L Q D N E L

FIG. 2A

1021 TGCCGGGCTGGATGAAGACCAGCGCCTGCCCTGCATGCCCAGAAATTTCCACAAGCAGC 1080  
P G L D E R P A P A L H A Q N F H K H E  
1081 AGTTCTGGTCCAGTCCTTTCGGGCACTGAACCCCGCACGGCGGCAAGACCGCAAGA 1140  
F W S S P F R A L K P R T A R K D R K K  
1141 AGAAGGACCAGGACACATTCAACCGCCGCTCCTCAGGTGCTGGACTTTGACGAGAAGA 1200  
K D Q D T F T A A S S Q V L D F D E K T  
1201 CGATGCAGAAAGCCAGGAGCGGAGTGGATGAGCCCGGCTGCTCCAGGAGGTACC 1260  
M Q K A R R R Q W D E P R V C S R R Y L  
1261 TGAAGGTGGATTTGCGAGACATCGGGTGAATGAATGGATCATCTCTCCCAAATCCTTGG 1320  
K V D F A D I G W N E W I I S P K S F D  
1321 ACGCCTACTACTGTGGGCGCTCGAGTTCGCCATGCCCAAGATTGTCCGCCCATCCA 1380  
A Y Y C A G A C E F P M P K I V R P S N  
1381 ACCATGCCACCATCCAGAGCATCGTCAGAGCTGTGGGCATTGTCCCTGGCATCCAGAGC 1440  
H A T I Q S I V R A V G I V P G I P E P  
1441 CATGCTGTGTTCCAGACAAGATGAACCTCCCTGGAGTCCTTTTCCTGGATGAAATCGGA 1500  
C C V P D K M N S L G V L F L D E N R N  
1501 ATGCGGTTCTGAAGGTGTACCCCAATATGTCCGTAGAGACCTGTGCTGCGTAAGATG 1560  
A V L K V Y P N M S V E T C A C R \*  
1561 GCTTCAAGATAGAACAGACAGACCTGTCTTCATCCCTGCCCTGCAGAGTGGCAATCTTGGAGC 1620  
1621 CAGGACTTGACTCGGGAGGTTCCAGGTGCTAGACAGAGCTTACAGGCAGCCCTGCTGG 1680  
1681 GACCAAGAAAGATCTGCCCAACACATCGCAATCTTCAGTTCTCCGTGCTGGGTAGC 1740  
1741 TCTGTAAGACGTGTGAGTTCCTGGAAGAAATCTGGAAATTAAGTGTGCTGCAATTTG 1800  
1801 CCCATCATCCCTGCCACACATTTTCAAGGCCTAGAAATAACGTGTGCTCCTCAAAATGTCAA 1860  
1861 CTCCAGGCATTTGTCTCTCAAAACCTAGAAAGACTATGCAAAATCTTGGGGTACTCCCCC 1920  
1921 CCCCCATGGCAGTTTAAATGCTGTTTAAACCCCTCAGGCTGCATTCTAGAAACAGGGCC 1980  
1981 TAACCCATGGCAGGTGAGTATTTCTCTTACGTTTCACTACACGTGCTTTTATACATG 2040  
2041 CAGTATGCACATGTAATCACGGTTGATTTCTTCTTTTAAATATATGATTTCTATTTCAAA 2100  
2101 GCAAAACGGAGAGTCGATCCCATCCCTGCAGAGGTAATAATGCAAGTTAGGTGTGGG 2160  
2161 TTGTCTAAGCATGTGTATGGAAATAATACATACAGTAATATGCTGGAATACTAAAAAGT 2220  
2221 AACCAAGATTTTATATTTTGTAAATTA TACTTTGTATACTGTAGATTGTGAGTGTCTG 2280  
2281 TGTTTTATGGAAAGCTAATAAATTAAAGGTGCGGAGGTATC 2322

FIG. 2B



GDF-10	EKSMQKARRRQWDEPRVCSRRYLKVDF-ADIGWNEWIISPKSFDAYYCAGACEFPMPKIVRPS--
GDF-1	RPRRDAEPVLGGPGGACRARRLYVSF-REVGWHRWVIAPRGFLANYCQGCQCALPVALSGSGGPP
GDF-3	RKRRAAISVPKGFCRNFCRRHQLFINF-QDLGWHKKWVIAPKGFMANYPCHGECPFSMTTYLNS---
GDF-9	SFNLSEYFKQFLFPQNECELHDFRLSF-SQLKWDNWIIVAPHRYNPRYCKGDCPPRAVRHRYGS---
BMP-2	REKRQAKHKQKRRLKSSCKRHPLYVDF-SDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNS---
BMP-4	KRSPKHHSQARAKKNKNCRRHSLYVDF-SDVGWNDWIVAPPGYQAFYCHGDCPPFPLADHLNS---
Vgr-1	SRGSGSDYNGSELKTAACKKHELYVSF-QDLGWQDWIIAPKGYAANYCDGECSPFLNAHMNA---
OP-1	LRMANVAENSSDQORQACKKHELYVSF-RDLGWQDWIIAPEGYAANYCEGECAPFLNSYMNA---
BMP-5	SRMSSVGDYNTSEQKQACKKHELYVSF-RDLGWQDWIIAPEGYAANYCDGECSPFLNAHMNA---
OP-2	RLPGIFDDVHGSHGRQVCRRHELYVSF-QDLGWLDWVIAPQGYSAAYCEGECSPFLDSCMNA---
BMP-3	EQTLKKARRKQWIEPRNCAARRYLKVDF-ADIGWSEWIIISPKSFDAYYCAGACEFPMPKSLKPS---
MIS	GPGRAQRSAGATAADGPCALRELSVDL---RAERSVLIPETYQANNQGVCGWPQSDRNPY---
Inhibin $\alpha$	ALRLLQRPPEEPAAHANCRRVALNISF-QELGWERWIVYPPSFIFHYCHGCCGLHIPNLSLPV-
Inhibin $\beta A$	RRRRRGLCEDGKV--NICKKQFFVSF-KDIGWNDWIIAPSGYHANYCEGECPSHIAGTSGSSL-
Inhibin $\beta B$	RIRKRGLCEDGRT--NLCCRQQFFIDF-RLIGWNDWIIAPTGYGNYCEGSCPAYLAGVPGSAS-
Nodal	GWGRRQRHHLPDRSQLCRRVKFQVDF-NLIGWGSWIIYPKQYNAYRCEGECNPNVGEFHP---
TGF- $\beta 1$	RRALDTNYCFSSSTE-KNCCVRQLYIDFRKDLGWK-WIHEPKGYHANFCLGFCOPYIWSLD-----
TGF- $\beta 2$	KRALDAAYCFRNVQ-DNCCLRPLYIDFKRDLGWK-WIHEPKGYNANFACAGACPYLWSSD-----
TGF- $\beta 3$	KRALDTNYCFRNLE-ENCCVRPLYIDFRQDLGWK-WVHEPKGYANFCSGFCOPYLRSAD-----

FIG. 3A

GDF-10	--NHATIQSIVRA-VGIVPGIPEPCCV--PDKMNSLGVFL-DENRNAVLKVYPNMSVETCAOR
GDF-1	ALNHAVLRALMHA--APGAADLPCCV--PARLSPISVLFF-DNSDNVVLRQYEDMVVDECCOR
GDF-3	-SNYAFMQALMHM--ADPKVPKAVCV--PTKLSPISMLYQ-DSDKNVILRHYEDMVVDECCCG
GDF-9	-PVHTMVQNIYE--KLDPSVPRPSCV--PGKYSPLSVLTI-EPDGSIAKEYEDMIATPCTICR
BMP-2	-TNHAIIVQTLVNS--VNSKIIPKACCV--PTELSAISMLYL-DENEKVVVLKNYQDMVVECCOR
BMP-4	-TNHAIIVQTLVNS--VNSSIIPKACCV--PTELSAISMLYL-DEYDKVVVLKNYQEMVVECCOR
Vgr-1	-TNHAIIVQTLVHL--MNPEYVPKPCA--PTKLNAISVLYF-DDNSNVILKKYRNMMVVRACCH
OP-1	-TNHAIIVQTLVHF--INPETVPKPCA--PTQLNAISVLYF-DDSSNVILKKYRNMMVVRACCH
BMP-5	-TNHAIIVQTLVHL--MFPDHVPKPCA--PTKLNAISVLYF-DDSSNVILKKYRNMMVVRACCH
OP-2	-TNHAILQSLVHL--MKPNAVPKACA--PTKLSATSVLYY-DSSNVILRKARNMVVKACCH
BMP-3	--NHATIQSIVRA-VGVVPGIPEPCCV--PEKMSSLSILFF-DENKNVVLKVYPNMTVESOACR
MIS	-GNHVLLKMQA--RGAALARPPCCV--PTAYAGKLLISLSEER--ISAHVVPNMVATECCOR
Inhibin $\alpha$	-PGAPPTPAQPYS--LLPGAQPCCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQHACI
Inhibin $\beta$ A	-SFHSTVINHYRMRGHSPFANLKSCCV--PTKLRPMSMLY-DDGQNIKKDIQNMIVECCOS
Inhibin $\beta$ B	-SFHTAVVNQYRMGLNPGT-VNSCCI--PTKLSTMSMLYF-DDEYNIVKRDVPNMIVECCCA
Nodal	-TNHAYIQSLLKR--YQPHRVPSTCCA--PVKTKPLSMLYV--DNGRVLLEHHKDMIVECCCL
TGF- $\beta$ 1	-TOYSKVLALYNQ--HNPGASAAPCCV--PQALEPLPIVYV-VGRKPKV-EQLSNMIVRSCKOS
TGF- $\beta$ 2	-TQHSRVLSLYNT--INPEASASPCCV--SQDLEPLTILYV-IGKTPKI-EQLSNMIVKSKOS
TGF- $\beta$ 3	-TTHSTVLGLYNT--LNPEASASPCCV--PQDLEPLTILYV-VGRTPKV-EQLSNMVVKSKOS

FIG. 3B

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% amino acid  
identity with  
GDF-10

GDF-1	38%
GDF-3	37%
GDF-9	28%
BMP-2	46%
BMP-4	45%
Vgr-1	43%
OP-1	41%
BMP-5	41%
OP-2	39%
BMP-3	83%
MIS	31%
Inhibin $\alpha$	28%
Inhibin $\beta$ A	36%
Inhibin $\beta$ B	35%
Nodal	40%
TGF- $\beta$ 1	30%
TGF- $\beta$ 2	30%
TGF- $\beta$ 3	29%

FIG. 4

KARRKQWDEPRVCSRRYLKVDFADIGWNEWII SPKSFDAYYCAGACEFPM  
 ||||:|||||H|||||||  
 KARRRQWDEPRVCSRRYLKVDFADIGWNEWII SPKSFDAYYCAGACEFPM  
 PKIVRPSNHATIQSIVRAVGII PG IPEPCCVPDKMNSLGVLFLDENRNVV  
 |||||:|||||  
 PKIVRPSNHATIQSIVRAVGIVPG IPEPCCVPDKMNSLGVLFLDENRNAV  
 LKVYPNMSVDTCACR  
 |||||:|||||  
 LKVYPNMSVETCACR

**FIG. 5**

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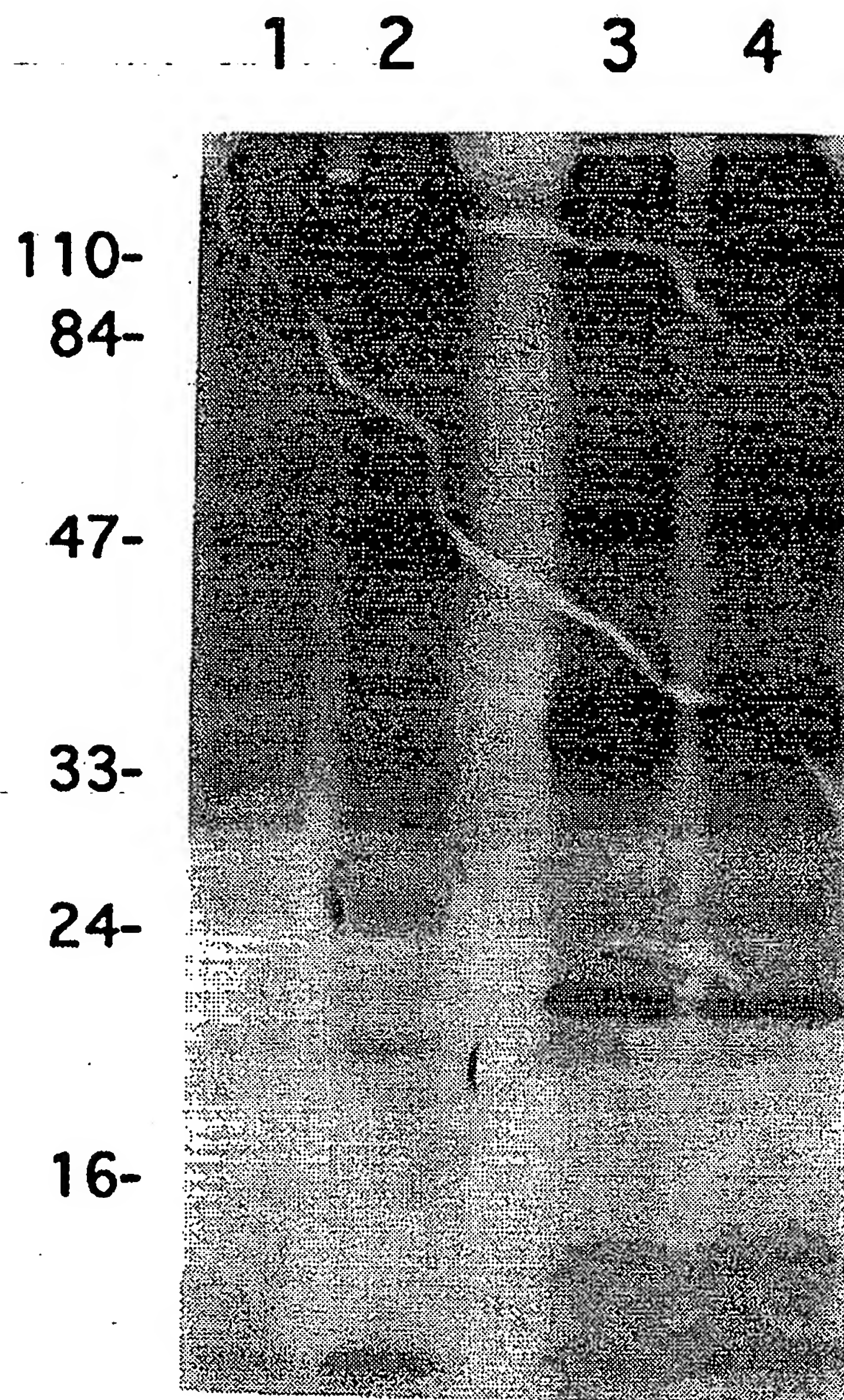


FIG. 6



**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :C07K 14/71; C07H 21/00

US CL :530/399; 536/23.5; 435/69.1, 69.4, 320.1, 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399; 536/23.5; 435/69.1, 69.4, 320.1, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GenBank, APS, Dialog

search terms: GDF, endometriosis, uterine, pregnancy, cancer, malignancy

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Sciences USA, Volume 88, issued May 1991, S. Lee, "Expression of growth/differentiation factor 1 in the nervous system: Conservation of a bicistronic structure", pages 4250-4254.	1-10
A	Journal of Biological Chemistry, Volume 268, No. 5, issued 15 February 1993, A.C. McPherron et al., "GDF-3 and GDF-9: Two members of the Transforming Growth Factor- $\beta$ Superfamily Containing a Novel Pattern of Cysteines", pages 3444-3449.	1-10

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
02 DECEMBER 1994	JAN 25 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer SHELLY GUEST CERMAK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Molecular Endocrinology, Volume 6, No. 11, issued 1992, C.M. Jones et al., "Isolation of <i>Vgr-2</i> , a Novel Member of the Transforming Growth Factor- $\beta$ -Related Gene Family", pages 1961-1968.	1-10
A	Molecular Endocrinology, Volume 4, No. 7, issued 1990, S. Lee, "Identification of a Novel Member (GDF-1) of the Transforming Growth Factor- $\beta$ Superfamily", pages 1034-1039.	1-10

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
€
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-10

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-10, drawn to a GDF protein and the DNA encoding the GDF-10 protein.

Group II, claims 11-44, drawn to an antibody and methods of using the antibody.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I and II are drawn to structurally distinct molecules, and although the antibody and GDF-10 protein are related immunochemically, the inventions are considered independent and distinct because they are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

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